

CLAIMS

WHAT IS CLAIMED IS:

1. A method for cloning non-natural and natural restriction endonucleases with co-expression of DNA ligase, comprising the steps of:

- a) preparing a first plasmid containing a gene encoding a DNA ligase;
- b) transfecting cells with the first plasmid so that DNA ligase is produced;
- c) preparing a second compatible plasmid containing a gene encoding a hybrid restriction endonuclease;
- d) transfecting said cells with said second plasmid; and
- e) cloning said cells.

2. The method of claim 1, wherein said cells are prokaryotic cells.

3. The method of claim 2, wherein said cells are *E. coli* cells.

4. The method of claim 1, wherein said cells are eukaryotic cells.

- 60 - 65

66

5. The method of claim 4, wherein said cells are plant cells.

6. The method of claim 4, wherein said cells are mammalian cells.

7. The method of claim 1, wherein said cells are mutant or engineered strains of cells that produce increased levels of DNA ligase.

8. The method of claim 1, wherein said gene encoding a hybrid restriction endonuclease is selected from the group consisting of ZF-QDR-F_N, ZF-Sp1C-F_N, ZF-QNR-F_N, ZF-QQR-F_N and ZFHD1-F_N.

9. A method for enzymatically inactivating a target DNA, comprising the steps of:

a) preparing a plasmid, phage, virus or any other delivery vehicle such as a liposome containing a gene encoding a nuclease, wherein said nuclease specifically recognizes and enzymatically inactivates said target DNA;

b) delivering the plasmid, phage, virus or any other delivery vehicle such as a liposome containing the gene encoding a nuclease into cells;

c) inducing said cells to produce said nuclease; and

d) enzymatically inactivating said target DNA.

10. The method of claim 9, wherein the gene encoding a nuclease is delivered into cells by way of liposomes.

*Step A2
cont*

11. The method of claim 9, wherein said delivering step further comprises integrating the gene encoding a nuclease into a chromosome of said cells.

12. The method of claim 9, wherein the gene encoding a nuclease further comprises control elements.

13. The method of claim 9, wherein said cells are prokaryotic cells.

14. The method of claim 13, wherein said cells are *E. coli* cells.

15. The method of claim 9, wherein said cells are eukaryotic cells.

16. The method of claim 15, wherein said cells are plant cells.

SPN 2 *and* *17*

17. The method of claim 15, wherein said cells are mammalian cells.

18

18. The method of claim 9, wherein said nuclease is a naturally occurring restriction endonuclease.

SPN 3

19. The method of claim 9, wherein said nuclease is a non-naturally occurring restriction enzyme.

20. The method of claim 19, wherein said nuclease is a hybrid restriction endonuclease.

21. The method of claim 20, wherein said gene encoding said nuclease is selected from the group consisting of ZF-QDR-F_N, ZF-Sp1C-F_N, ZF-QNR-F_N, ZF-QQR-F_N and ZFHD1-F_N.

22. The method of claim 9, wherein said target DNA is a DNA exogenous to DNA of said cells.

23. The method of claim 22, wherein said target DNA is any self-replicating DNA, linear or circular.

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24. The method of claim 22, wherein said target DNA is a DNA intermediate of an RNA tumor virus.

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25. The method of claim 9, wherein said target DNA is a DNA endogenous to DNA of said cells.

26. The method of claim 25, wherein the target DNA is chromosomal DNA of said cells.

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